

=> d his

(FILE 'HOME' ENTERED AT 10:57:30 ON 20 FEB 2007)

FILE 'CAPLUS' ENTERED AT 10:57:42 ON 20 FEB 2007

L1 53660 S PHOSPHOPROTEIN OR PHOSPHOPEPTIDE OR (PHOSPHORYL#(L) (PROTEIN O
L2 1412 S L1(L) (ISOLAT# OR SEPARAT# OR CHROOMATOGRAPHY)
L3 89 S L2 AND (GALLIUM OR GA OR IRON OR FE OR ALUMINUM OR AL)
L4 7 S L3 AND (METAL(L)CHELAT# OR BAPTA OR IDA OR DTPA OR PHENANTHRO

=> d que l4 stat

L1 53660 SEA FILE=CAPLUS ABB=ON PLU=ON PHOSPHOPROTEIN OR PHOSPHOPEPTID
E OR (PHOSPHORYL#(L) (PROTEIN OR PEPTIDE OR AMINO ACID OR
CARBOHYDRATE OR LIPID OR PHOSPHATASE OR KINASE))
L2 1412 SEA FILE=CAPLUS ABB=ON PLU=ON L1(L) (ISOLAT# OR SEPARAT# OR
CHROOMATOGRAPHY)
L3 89 SEA FILE=CAPLUS ABB=ON PLU=ON L2 AND (GALLIUM OR GA OR IRON
OR FE OR ALUMINUM OR AL)
L4 7 SEA FILE=CAPLUS ABB=ON PLU=ON L3 AND (METAL(L)CHELAT# OR
BAPTA OR IDA OR DTPA OR PHENANTHROLINE)

=> d 1-7 bib abs

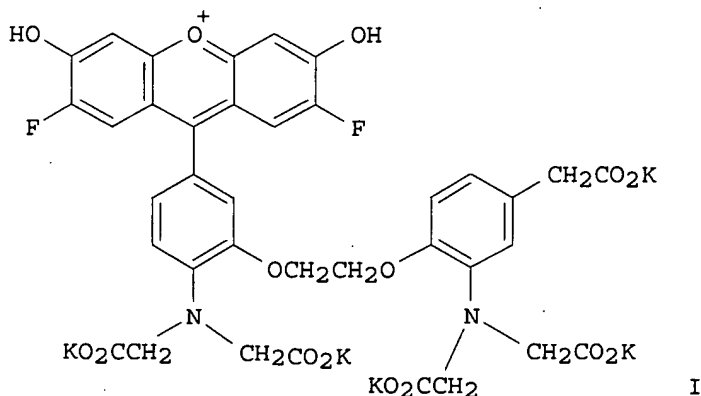
L4 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2006:1280218 CAPLUS
DN 146:137856
TI Magnetic IDA-modified hydrophilic methacrylate-based polymer
microspheres for IMAC protein separation
AU Prikryl, Petr; Horak, Daniel; Ticha, Marie; Kuceroval, Zdenka
CS Institute of Pathophysiology, 1st Faculty of Medicine and Centre of
Experimental Hematology, Charles University, Prague, Czech Rep.
SO Journal of Separation Science (2006), 29(16), 2541-2549
CODEN: JSSCCJ; ISSN: 1615-9306
PB Wiley-VCH Verlag GmbH & Co. KGaA
DT Journal
LA English
AB Preparation of a new type of magnetic non-porous poly(2-hydroxyethyl
methacrylate-co-glycidyl methacrylate) microspheres with hydrophilic
properties containing coupled iminodiacetic acid (IDA) is described.
The prepared microspheres were used for the immobilization of Ni(II) or
Fe(III) ions to show their application in protein binding studies.
Human IgG was bound to magnetic Ni(II)-IDA-modified microspheres
and conditions of its adsorption and elution were optimized. Non-specific
binding of the protein to magnetic microspheres in the absence of Ni(II)
ions was low. Fe(III) ions immobilized on magnetic IDA
-modified microspheres were used for the specific binding of porcine
pepsin, as a model phosphoprotein. The ability of phosphate
buffer to release the adsorbed enzyme from the microspheres and a low
adsorption of the dephosphorylated protein indicate the participation of
phosphate groups in the pepsin interaction. The elaborated method
represents a rapid technique that can be used not only for the
separation of proteins but also for anal. purposes.
RE.CNT 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2005:532184 CAPLUS
DN 143:149205
TI Characterisation and evaluation of metal-loaded iminodiacetic acid-silica
of different porosity for the selective enrichment of phosphopeptides
AU Trojer, L.; Stecher, G.; Feuerstein, I.; Lubbad, S.; Bonn, G. K.
CS Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens
University, Innsbruck, 6020, Austria
SO Journal of Chromatography, A (2005), 1079(1-2), 197-207
CODEN: JCRAEY; ISSN: 0021-9673
PB Elsevier B.V.
DT Journal
LA English
AB Silica particles of different porosity were functionalized with
iminodiacetic acid (IDA) and loaded with Fe(III) to
yield immobilized metal affinity chromatog. stationary phases (Fe
(III)-IDA-silica) for phosphopeptide enrichment. The
elution step of bound phosphopeptides was optimized with a ³²P
radioactive labeled peptide by a comprehensive study. Several elution
systems, including phosphate buffers of different pH and concentration and EDTA
solns. were employed. Furthermore the effect of support porosity on
elution behavior was investigated. Under best conditions recoveries
higher than 90% were achieved. A solid-phase extraction (SPE) protocol was
developed for fractionation of phosphorylated and non-phosphorylated
peptides and desalting of the fractions which is essential for subsequent
mass spectrometric anal. by the combination of Fe(III)-
IDA-silica and C18-silica particles. The pH of the loading buffer
was a critical parameter for the efficiency of the SPE protocol. As tryptic
digests of α -lactalbumin, lysozyme and RNase A mixed with three
synthetic phosphopeptides were fractionated, pH 2.5 provided
minimal proportion of unspecific bound peptides when comparing the
fractions after μ -LC-electrospray ionization MS separation. The
effect of a sample derivatization reaction (methylation) on the efficiency
of phosphopeptide enrichment was further investigated. Blocking
carboxylate groups by Me ester formation totally prevented unspecific
interaction with the immobilized Fe(III) ions, but generated
partially methylated phosphopeptides that increased the
complexity of the phosphorylated fraction.
RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2005:146907 CAPLUS
DN 143:111777
TI Application of metal-chelate affinity chromatography
to the study of the phosphoproteome
AU Imam-Sghiouar, N.; Joubert-Caron, R.; Caron, M.
CS Laboratoire de Biochimie des Proteines et Proteomique, EA 3408, UFR SMBH
Leonard de Vinci, Bobigny, Fr.
SO Amino Acids (2005), 28(1), 105-109
CODEN: AACIE6; ISSN: 0939-4451
PB Springer Wien
DT Journal
LA English
AB With the increasing importance of proteome anal., studying the
phosphoproteome is a priority for functional studies. Therefore, a
rational approach to simplifying the proteome is needed. In this work, we
examined the use of immobilized metal affinity chromatog. (IMAC) using
ferric ions-chelated column for enriching crude cell exts. in
phosphoproteins. The adsorption of the proteins on Fe³⁺ was
obtained at an acidic pH 5.6, and their elution at a more basic pH in Tris
buffer. To evaluate the separation, western blots were performed
with either anti-phosphotyrosine or anti-phosphoserine/threonine. The
anal. of the eluates demonstrated the selectivity of the separation,
particularly for proteins phosphorylated on serine or threonine. In
conclusion, the advantages and the limits of this approach are discussed.
RE.CNT 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN *APPLICANT*
 AN 2005:58103 CAPLUS
 DN 142:130341
 TI Metal-binding molecules and metal complexes and methods for detection and isolation of phosphorylated molecules
 IN Agnew, Brian; Gee, Kyle R.; Martin, Vladimir V.
 PA USA
 SO U.S. Pat. Appl. Publ., 96 pp., Cont.-in-part of U.S. Ser. No. 703,816.
 CODEN: USXXCO
 DT Patent
 LA English
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2005014197	A1	20050120	US 2004-821522	20040409
	US 2004038306	A1	20040226	US 2003-428192	20030502
	US 7102005	B2	20060905		
	CA 2483868	A1	20040521	CA 2003-2483868	20030502
	AU 2003299466	A1	20040607	AU 2003-299466	20030502
	EP 1546118	A2	20050629	EP 2003-799756	20030502
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
	JP 2005539243	T	20051222	JP 2004-549877	20030502
	US 2004171034	A1	20040902	US 2003-703816	20031107
PRAI	US 2002-377733P	P	20020503		
	US 2002-393059P	P	20020628		
	US 2002-407255P	P	20020830		
	US 2003-440252P	P	20030114		
	US 2003-428192	A2	20030502		
	US 2003-703816	A2	20031107		
	WO 2003-US13765	W	20030502		
OS	MARPAT 142:130341				
GI					

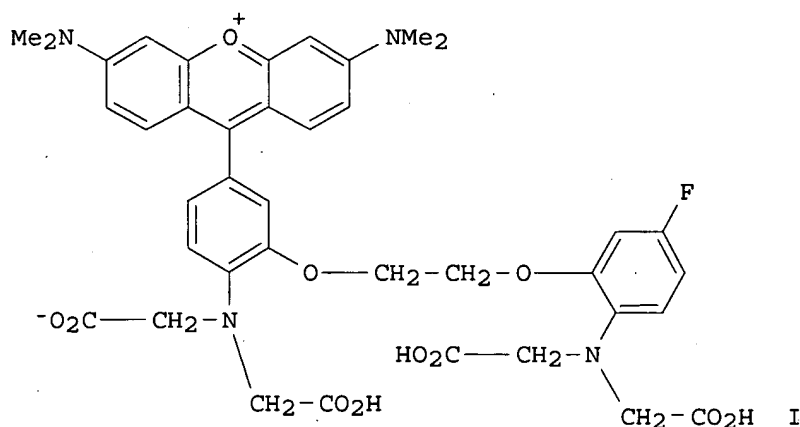


AB The present invention relates to phosphate-binding compds. that find use in binding, detecting and isolating phosphorylated target mols. including the subsequent identification of target mols. that interact with phosphorylated target mols. or mols. capable of being phosphorylated. The phosphate-binding compds. comprise a metal-chelating moiety such as BAPTA, DTPA, IDA, and phenanthroline. This metal-chelating moiety is desirably attached to a label, e.g., a dye or a hapten and/or a reactive group. Preferred dyes are benzofurans, quinazolinones, xanthenes, indoles, benzazoles, and borapolyazaindacenes. A binding solution is provided that comprises a phosphate-binding compound, an

acid and a metal ion wherein the metal ion simultaneously interacts with an exposed phosphate group on a target mol. and the metal chelating moiety of the phosphate-binding compound forming a bridge between the phosphate-binding compound and a phosphorylated target mol. resulting in a ternary complex. The binding solution of the present invention finds use in binding and detecting immobilized and solubilized phosphorylated target mols., isolation of phosphorylated target mols. from a complex mixture and aiding in proteomic anal. wherein kinase and phosphatase substrates and enzymes can be identified. Thus, a compound comprising dihydroxydifluoroxanthene attached to BAPTA and dextran (I) was prepared. I might be used, after addition of GaCl_3 to form complexes, as an affinity matrix to isolate phosphopeptides. The phosphopeptides might then be identified by mass spectrometry.

L4 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
 AN 2004:162337 CAPLUS
 DN 140:213577
 TI Compositions and methods for detection and isolation of phosphorylated molecules
 IN Agnew, Brian; Beechem, Joseph; Gee, Kyle; Haugland, Richard; Liu, Jixiang; Martin, Vladimir; Patton, Wayne; Steinberg, Thomas
 PA Molecular Probes, Inc., USA
 SO U.S. Pat. Appl. Publ., 83 pp.
 CODEN: USXXCO
 DT Patent
 LA English
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2004038306	A1	20040226	US 2003-428192	20030502
	US 7102005	B2	20060905		
	CA 2483868	A1	20040521	CA 2003-2483868	20030502
	WO 2004042347	A2	20040521	WO 2003-US13765	20030502
	WO 2004042347	A3	20050414		
	W:				
	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW:				
	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU	2003299466	A1	20040607	AU 2003-299466	20030502
EP	1546118	A2	20050629	EP 2003-799756	20030502
	R:				
	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
CN	1665790	A	20050907	CN 2003-815684	20030502
JP	2005539243	T	20051222	JP 2004-549877	20030502
US	2004171034	A1	20040902	US 2003-703816	20031107
US	2005014197	A1	20050120	US 2004-821522	20040409
PRAI	US 2002-377733P	P	20020503		
	US 2002-393059P	P	20020628		
	US 2002-407255P	P	20020830		
	US 2003-440252P	P	20030114		
	US 2003-428192	A2	20030502		
	WO 2003-US13765	W	20030502		
	US 2003-703816	A2	20031107		
OS	MARPAT 140:213577				
GI					



AB The present invention relates to phosphate-binding compds. that find use in binding, detecting and isolating phosphorylated target mols. including the subsequent identification of target mols. that interact with phosphorylated target mols. or mols. capable of being phosphorylated. A binding solution is provide that comprises a phosphate-binding compound, an acid and a metal ion wherein the metal ion simultaneously interacts with an exposed phosphate group on a target mol. and the metal chelating moiety of the phosphate-binding compound forming a bridge between the phosphate-binding compound and a phosphorylated target mol. resulting in a ternary complex. The binding solution of the present invention finds use in binding and detecting immobilized and solubilized phosphorylated target mols., isolation of phosphorylated target mols. from a complex mixture and aiding in proteomic anal. wherein kinase and phosphatase substrates and enzymes can be identified. A human MRC-5 lung fibroblast cell lysate protein mixture was separated by two-dimensional gel electrophoresis. The gel was fixed and then phosphoproteins were stained with a solution containing 50 mM NaOAc, pH 4.0, 250 mM NaCl, 20% volume/volume 1,2-propanediol, 1 μ M rhodamine- BAPTA chelating compound I, and 1 μ M gallium chloride.

RE.CNT 208 THERE ARE 208 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN

AN 1991:97590 CAPLUS

DN 114:97590

TI Rapid metal-interaction chromatography of proteins and peptides on micropellicular sorbents

AU Bonn, G. K.; Kalghatgi, K.; Horne, W. C.; Horvath, C.

CS Inst. Radiochem., Univ. Innsbruck, Innsbruck, A-6020, Austria

SO Chromatographia (1990), 30(9-10), 484-8

CODEN: CHRGB7; ISSN: 0009-5893

DT Journal

LA English

AB Short columns packed with micropellicular stationary phases consisting of 2- μ m fused silica microspheres with covalently bound iminodiacetate (IDA) functions at the surface were used for rapid HPLC anal. of proteins by metal-interaction chromatog. (MIC). In contrast to conventional porous stationary phases which elicit relatively long anal. times, the columns packed with sorbents having micropellicular configuration and Ni²⁺ or Co²⁺ chelated by the IDA functions yielded separation of model proteins in a few minutes with good resolution. A Fe³⁺/IDA column was used for separation of phosphorylated and nonphosphorylated peptides derived from enzymically digested erythrocyte membrane proteins. Stability of the Fe³⁺/IDA column was quite satisfactory as determined by monitoring the iron content of the column effluent and by measuring the amount of iron present in the stationary phase.

L4 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
AN 1988:507179 CAPLUS
DN 109:107179
TI Immobilized metal ion affinity chromatography of proteins on IDA
-iron(3+)
AU Sulkowski, Eugene
CS Dep. Mol. Cell. Biol., Roswell Park Mem. Inst., Buffalo, NY, 14263, USA
SO Makromolekulare Chemie, Macromolecular Symposia (1988), 17(Int. Symp.
Affinity Chromatogr. Interfacial Macromol. Interact., 1987), 335-48
CODEN: MCMSES; ISSN: 0258-0322
DT Journal
LA English
AB Several proteins, selected for their varied isoelec. points within the pH
range .apprx.4 to .apprx.11, bind to immobilized (chelated to
iminodiacetate) ferric ion, IDA-Fe³⁺, when applied in a 50 mM
buffer (pH 6.0). These proteins can be displaced from IDA-Fe³⁺
columns by an increase of pH from 6 to 8, an increase of NaCl from 0 to
1M, by both. Apotransferrins, in contrast to other proteins, are able to
scavenge Fe³⁺ from IDA-Fe³⁺. Two proteins, both quite acidic,
behave quite differently on IDA-Fe³⁺: α 1-acid glycoprotein
(sialic acid) does not bind, whereas phosvitin (phosphate!) binds avidly.
IDA-Fe³⁺ sorbent, due to its unusual sorptive properties,
represents a new addition of particular significance to the family of
chromatog. sorbents available for protein purification

=> => d que 18 stat

L5	22	SEA FILE=CAPLUS ABB=ON	PLU=ON	("AGNEW BRIAN"/AU OR "AGNEW BRIAN J"/AU)
L6	94	SEA FILE=CAPLUS ABB=ON	PLU=ON	("GEE KYLE"/AU OR "GEE KYLE R"/AU)
L7	25	SEA FILE=CAPLUS ABB=ON	PLU=ON	("MARTIN VLADIMIR"/AU OR "MARTIN VLADIMIR V"/AU)
L8	126	SEA FILE=CAPLUS ABB=ON	PLU=ON	L5 OR L6 OR L7

=> d que 19 stat

L5	22	SEA FILE=CAPLUS ABB=ON	PLU=ON	("AGNEW BRIAN"/AU OR "AGNEW BRIAN J"/AU)
L6	94	SEA FILE=CAPLUS ABB=ON	PLU=ON	("GEE KYLE"/AU OR "GEE KYLE R"/AU)
L7	25	SEA FILE=CAPLUS ABB=ON	PLU=ON	("MARTIN VLADIMIR"/AU OR "MARTIN VLADIMIR V"/AU)
L8	126	SEA FILE=CAPLUS ABB=ON	PLU=ON	L5 OR L6 OR L7
L9	1	SEA FILE=CAPLUS ABB=ON	PLU=ON	L8 AND PHOSPHO#

=> d bib abs

L9 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2007 ACS on STN
 AN 2005:472425 CAPLUS
 DN 143:22621
 TI Competitive immunoassay using a ligand analog covalently bonded to a
 fluorescent reporter molecule
 IN Beechem, Joseph; Gee, Kyle; Hagen, David; Johnson, Iain; Kang,
 Hee Chol; Pastula, Christina
 PA Molecular Probes, Inc., USA
 SO PCT Int. Appl., 123 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2005050206	A2	20050602	WO 2004-US30711	20040917
	WO 2005050206	A3	20060302		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW			
	RW:	BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	US 2006160068	A1	20060720	US 2004-943463	20040917
PRAI	US 2003-504322P	P	20030917		
	US 2003-505455P	P	20030923		

OS MARPAT 143:22621

AB The present invention provides ligand-detection reagents, ligand analogs and methods for determining the presence of a ligand in a sample. The ligand-detection reagent comprises a ligand-binding antibody and a ligand analog to form an antibody-ligand analog complex wherein the ligand analog is covalently bonded to a reporter mol. This complex may addnl. comprise a labeling protein non-covalently bonded to the antibody to form a ternary complex wherein the labeling protein comprises a monovalent antibody fragment or a non-antibody protein that is covalently bonded to a label moiety. The reporter mol. is either quenched by the ligand-binding antibody or by the label moiety of the labeling protein, depending on the reporter mol. and the ligand-binding antibody, wherein the amount of quenching is directly related to the amount of ligand present in the sample. Alternatively, the ligand analog is fluorogenic wherein the ligand analog is essentially non-fluorescent in solution but when bound by the ligand-binding antibody the detectable signal increases. In this instance a decrease in signal, as opposed to the relieving of quenching, is measured for the presence of a target ligand.

=> s 18 and phosphorylated

55075 PHOSPHORYLATED

L10 9 L8 AND PHOSPHORYLATED

=> d 1-9 bib abs

L10 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN
 AN 2005:472425 CAPLUS
 DN 143:22621
 TI Competitive immunoassay using a ligand analog covalently bonded to a
 fluorescent reporter molecule
 IN Beechem, Joseph; Gee, Kyle; Hagen, David; Johnson, Iain; Kang,
 Hee Chol; Pastula, Christina
 PA Molecular Probes, Inc., USA
 SO PCT Int. Appl., 123 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 1

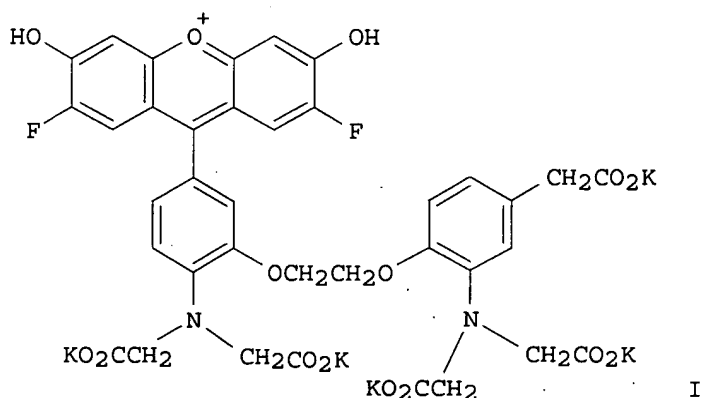
	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2005050206	A2	20050602	WO 2004-US30711	20040917
	WO 2005050206	A3	20060302		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW			
	RW:	BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	US 2006160068	A1	20060720	US 2004-943463	20040917
PRAI	US 2003-504322P	P	20030917		
	US 2003-505455P	P	20030923		

OS MARPAT 143:22621

AB The present invention provides ligand-detection reagents, ligand analogs and methods for determining the presence of a ligand in a sample. The ligand-detection reagent comprises a ligand-binding antibody and a ligand analog to form an antibody-ligand analog complex wherein the ligand analog is covalently bonded to a reporter mol. This complex may addnl. comprise a labeling protein non-covalently bonded to the antibody to form a ternary complex wherein the labeling protein comprises a monovalent antibody fragment or a non-antibody protein that is covalently bonded to a label moiety. The reporter mol. is either quenched by the ligand-binding antibody or by the label moiety of the labeling protein, depending on the reporter mol. and the ligand-binding antibody, wherein the amount of quenching is directly related to the amount of ligand present in the sample. Alternatively, the ligand analog is fluorogenic wherein the ligand analog is essentially non-fluorescent in solution but when bound by the ligand-binding antibody the detectable signal increases. In this instance a decrease in signal, as opposed to the relieving of quenching, is measured for the presence of a target ligand.

L10 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN
 AN 2005:58103 CAPLUS
 DN 142:130341
 TI Metal-binding molecules and metal complexes and methods for detection and isolation of phosphorylated molecules
 IN Agnew, Brian; Gee, Kyle R.; Martin, Vladimir V.
 PA USA
 SO U.S. Pat. Appl. Publ., 96 pp., Cont.-in-part of U.S. Ser. No. 703,816.
 CODEN: USXXCO
 DT Patent
 LA English
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2005014197	A1	20050120	US 2004-821522	20040409
	US 2004038306	A1	20040226	US 2003-428192	20030502
	US 7102005	B2	20060905		
	CA 2483868	A1	20040521	CA 2003-2483868	20030502
	AU 2003299466	A1	20040607	AU 2003-299466	20030502
	EP 1546118	A2	20050629	EP 2003-799756	20030502
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
	JP 2005539243	T	20051222	JP 2004-549877	20030502
	US 2004171034	A1	20040902	US 2003-703816	20031107
PRAI	US 2002-377733P	P	20020503		
	US 2002-393059P	P	20020628		
	US 2002-407255P	P	20020830		
	US 2003-440252P	P	20030114		
	US 2003-428192	A2	20030502		
	US 2003-703816	A2	20031107		
	WO 2003-US13765	W	20030502		
OS	MARPAT 142:130341				
GI					



AB The present invention relates to phosphate-binding compds. that find use in binding, detecting and isolating phosphorylated target mols. including the subsequent identification of target mols. that interact with phosphorylated target mols. or mols. capable of being phosphorylated. The phosphate-binding compds. comprise a metal-chelating moiety such as BAPTA, DTPA, IDA, and phenanthroline. This metal-chelating moiety is desirably attached to a label, e.g., a dye or a

hapten and/or a reactive group. Preferred dyes are benzofurans, quinazolinones, xanthenes, indoles, benzazoles, and borapolyazaindacenes. A binding solution is provided that comprises a phosphate-binding compound, an acid and a metal ion wherein the metal ion simultaneously interacts with an exposed phosphate group on a target mol. and the metal chelating moiety of the phosphate-binding compound forming a bridge between the phosphate-binding compound and a phosphorylated target mol. resulting in a ternary complex. The binding solution of the present invention finds use in binding and detecting immobilized and solubilized phosphorylated target mols., isolation of phosphorylated target mols. from a complex mixture and aiding in proteomic anal. wherein kinase and phosphatase substrates and enzymes can be identified. Thus, a compound comprising dihydroxydifluoroxanthene attached to BAPTA and dextran (I) was prepared. I might be used, after addition of $GaCl_3$ to form complexes, as an affinity matrix to isolate phosphopeptides. The phosphopeptides might then be identified by mass spectrometry.

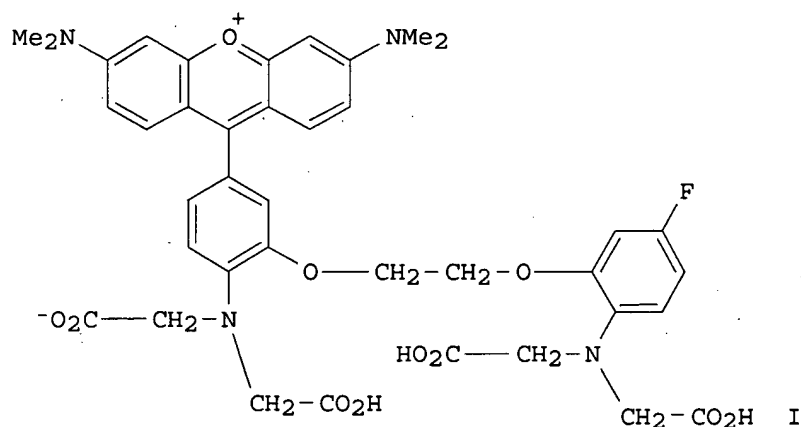
L10 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN
 AN 2004:722822 CAPLUS
 DN 141:239312
 TI Compositions and methods for detection and isolation of
 phosphorylated molecules
 IN Agnew, Brian; Beechem, Joseph; Gee, Kyle; Haugland,
 Richard; Steinberg, Thomas; Patton, Wayne
 PA USA
 SQ U.S. Pat. Appl. Publ., 89 pp., Cont.-in-part of U.S. Ser. No. 428,192.
 CODEN: USXXCO
 DT Patent
 LA English
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2004171034	A1	20040902	US 2003-703816	20031107
	US 2004038306	A1	20040226	US 2003-428192	20030502
	US 7102005	B2	20060905		
	CA 2483868	A1	20040521	CA 2003-2483868	20030502
	AU 2003299466	A1	20040607	AU 2003-299466	20030502
	EP 1546118	A2	20050629	EP 2003-799756	20030502
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	JP 2005539243	T	20051222	JP 2004-549877	20030502
	US 2005014197	A1	20050120	US 2004-821522	20040409
	WO 2005047901	A2	20050526	WO 2004-US36968	20041105
	WO 2005047901	A3	20050728		
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PRAI	US 2002-377733P	P	20020503		
	US 2002-393059P	P	20020628		
	US 2002-407255P	P	20020830		
	US 2003-440252P	P	20030114		
	US 2003-428192	A2	20030502		
	WO 2003-US13765	W	20030502		
	US 2003-703816	A2	20031107		

AB The present invention relates to phosphate-binding compds. that find use in binding, detecting and isolating phosphorylated target mols. including the subsequent identification of target mols. that interact with phosphorylated target mols. or mols. capable of being phosphorylated. A binding solution is provide that comprises a phosphate-binding compound, an acid and a metal ion wherein the metal ion simultaneously interacts with an exposed phosphate group on a target mol. and the metal chelating moiety of the phosphate-binding compound forming a bridge between the phosphate-binding compound and a phosphorylated target mol. resulting in a ternary complex. The binding solution of the present invention finds use in binding and detecting immobilized and solubilized phosphorylated target mols., isolation of phosphorylated target mols. from a complex mixture and aiding in proteomic anal. wherein kinase and phosphatase substrates and enzymes can be identified.

L10 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN
 AN 2004:162337 CAPLUS
 DN 140:213577
 TI Compositions and methods for detection and isolation of
 phosphorylated molecules
 IN Agnew, Brian; Beechem, Joseph; Gee, Kyle; Haugland,
 Richard; Liu, Jixiang; Martin, Vladimir; Patton, Wayne;
 Steinberg, Thomas
 PA Molecular Probes, Inc., USA
 SO U.S. Pat. Appl. Publ., 83 pp.
 CODEN: USXXCO
 DT Patent
 LA English
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2004038306	A1	20040226	US 2003-428192	20030502
	US 7102005	B2	20060905		
	CA 2483868	A1	20040521	CA 2003-2483868	20030502
	WO 2004042347	A2	20040521	WO 2003-US13765	20030502
	WO 2004042347	A3	20050414		
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	CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,				
	GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,				
	LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,				
	PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ,				
	UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW:				
	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,				
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	FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR,				
	BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2003299466	A1	20040607	AU 2003-299466	20030502
	EP 1546118	A2	20050629	EP 2003-799756	20030502
	R:				
	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				
	IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
	CN 1665790	A	20050907	CN 2003-815684	20030502
	JP 2005539243	T	20051222	JP 2004-549877	20030502
	US 2004171034	A1	20040902	US 2003-703816	20031107
	US 2005014197	A1	20050120	US 2004-821522	20040409
PRAI	US 2002-377733P	P	20020503		
	US 2002-393059P	P	20020628		
	US 2002-407255P	P	20020830		
	US 2003-440252P	P	20030114		
	US 2003-428192	A2	20030502		
	WO 2003-US13765	W	20030502		
	US 2003-703816	A2	20031107		
OS	MARPAT 140:213577				
GI					



AB The present invention relates to phosphate-binding compds. that find use in binding, detecting and isolating phosphorylated target mols. including the subsequent identification of target mols. that interact with phosphorylated target mols. or mols. capable of being phosphorylated. A binding solution is provide that comprises a phosphate-binding compound, an acid and a metal ion wherein the metal ion simultaneously interacts with an exposed phosphate group on a target mol. and the metal chelating moiety of the phosphate-binding compound forming a bridge between the phosphate-binding compound and a phosphorylated target mol. resulting in a ternary complex. The binding solution of the present invention finds use in binding and detecting immobilized and solubilized phosphorylated target mols., isolation of phosphorylated target mols. from a complex mixture and aiding in proteomic anal. wherein kinase and phosphatase substrates and enzymes can be identified. A human MRC-5 lung fibroblast cell lysate protein mixture was separated by two-dimensional gel electrophoresis. The gel was fixed and then phosphoproteins were stained with a solution containing 50 mM NaOAc, pH 4.0, 250 mM NaCl, 20% volume/volume 1,2-propanediol, 1 μ M rhodamine-BAPTA chelating compound I, and 1 μ M gallium chloride.

RE.CNT 208 THERE ARE 208 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2003:619840 CAPLUS

DN 139:257650

TI Quantitative analysis of protein phosphorylation status and protein kinase activity on microarrays using a novel fluorescent phosphorylation sensor dye

AU Martin, Karen; Steinberg, Thomas H.; Cooley, Laurie A.; Gee, Kyle R.; Beechem, Joseph M.; Patton, Wayne F.

CS Molecular Probes, Eugene, OR, USA

SO Proteomics (2003), 3(7), 1244-1255

CODEN: PROTC7; ISSN: 1615-9853

PB Wiley-VCH Verlag GmbH & Co. KGaA

DT Journal

LA English

AB Ultrasensitive detection of minute amts. of phosphorylated proteins and peptides is a key requirement for unraveling many of the most important signal transduction pathways in mammalian systems. Protein microarrays are potentially useful tools for sensitive screening of global protein expression and post-translational modifications, such as phosphorylation. However, the anal. of signaling pathways has been hampered by a lack of reagents capable of conveniently detecting the targets of protein kinases. Historically, phosphorylation detection methods have relied upon either radioisotopes ((γ -³²P)ATP(γ -³³P)ATP labeling) or phosphoamino acid-selective antibodies. Both of these methods suffer from relatively well-known shortcomings. In this study, a small mol. fluorophore phosphosensor technol. is described, referred to as Pro-Q Diamond dye, which is capable of ultrasensitive global detection and quantitation of phosphorylated amino acid residues in peptides and proteins displayed on microarrays. The utility of the fluorescent Pro-Q Diamond phosphosensor dye technol. is demonstrated using phosphoproteins and phosphopeptides as well as with protein kinase reactions performed in miniaturized microarray assay format. Instead of applying a phosphoamino acid-selective antibody labeled with a fluorescent or enzymic tag for detection, a small, fluorescent probe is employed as a universal sensor of phosphorylation status. The detection limit for phosphoproteins on a variety of different com. available protein array substrates was found to be 312-625 fg, depending upon the number of phosphate residues. Characterization of the enzymic phosphorylation of immobilized peptide targets with Pro-Q Diamond dye readily permits differentiation between specific and non-specific peptide labeling at picogram to subpicogram levels of detection sensitivity.

RE.CNT 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2003:619829 CAPLUS

DN 139:273181

TI Global quantitative phosphoprotein analysis using multiplexed proteomics technology

AU Steinberg, Thomas H.; Agnew, Brian J.; Gee, Kyle R.;
Leung, Wai-Yee; Goodman, Terrie; Schulenberg, Birte; Hendrickson, Jill;
Beechem, Joseph M.; Haugland, Richard P.; Patton, Wayne F.

CS Molecular Probes, Eugene, OR, USA

SO Proteomics (2003), 3(7), 1128-1144

CODEN: PROTC7; ISSN: 1615-9853

PB Wiley-VCH Verlag GmbH & Co. KGaA

DT Journal

LA English

AB Systematic parallel anal. of the phosphorylation status of networks of interacting proteins involved in the regulatory circuitry of cells and tissues is certain to drive research in the post-genomics era for many years to come. Reversible protein phosphorylation plays a critical regulatory role in a multitude of cellular processes, including alterations in signal transduction pathways related to oncogene and tumor suppressor gene products in cancer. While fluorescence detection methods are likely to offer the best solution to global protein quantitation in proteomics, to date, there has been no satisfactory method for the specific and reversible fluorescent detection of gel-separated phosphoproteins from complex samples. The newly developed Pro-Q Diamond phosphoprotein dye technol. is suitable for the fluorescent detection of phosphoserine-, phosphothreonine-, and phosphotyrosine-containing proteins directly in SDS (SDS)-polyacrylamide gels and two-dimensional (2-D) gels. Addnl., the technol. is appropriate for the determination of protein kinase and phosphatase substrate preference. Other macromols., such as DNA, RNA, and sulfated glycans, fail to be detected with Pro-Q Diamond dye. The staining procedure is rapid, simple to perform, readily reversible and fully compatible with modern microchem. anal. procedures, such as matrix-assisted laser desorption/ionizationtime of flight (MALDI-TOF) mass spectrometry. Pro-Q Diamond dye technol. can detect as little as 1-2 ng omagnetic nanoparticles conjugated with β -casein, a pentaphosphorylated protein, and 8 ng of pepsin, a monophosphorylated protein. Fluorescence signal intensity correlates with the number of phosphorylated residues on the protein. Through combination of Pro-Q Diamond phosphoprotein stain with SYPRO Ruby protein gel stain, Multiplexed Proteomics technol. permits quant., dichromatic fluorescence detection of proteins in 2-D gels. This evolving discovery platform allows the parallel determination of protein expression level changes and altered

post-translational modification patterns within a single 2-D gel experiment

The linear responses of the fluorescence dyes utilized, allow rigorous quantitation of changes over an unprecedented 500-1000-fold concentration

range.

RE.CNT 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2003:395448 CAPLUS

DN 140:124749

TI Strategies and solid-phase formats for the analysis of protein and peptide phosphorylation employing a novel fluorescent phosphorylation sensor dye

AU Martin, Karen; Steinberg, Thomas H.; Goodman, Terrie; Schulenberg, Birte; Kilgore, Jason A.; Gee, Kyle R.; Beechem, Joseph M.; Patton, Wayne F.

CS Molecular Probes, Inc., Eugene, OR, 97402, USA

SO Combinatorial Chemistry and High Throughput Screening (2003), 6(4), 331-339

CODEN: CCHSFU; ISSN: 1386-2073

PB Bentham Science Publishers Ltd.

DT Journal

LA English

AB Protein kinases represent one of the largest families of regulatory enzymes, with more than 2,000 of them being encoded for by the human genome. Many cellular processes are regulated by the reversible phosphorylation of proteins and upwards of 30% of the proteins comprising the eukaryotic proteome are likely to be phosphorylated at some point during their existence. In the past, anal. of global protein phosphorylation has been accomplished through radiolabelling of samples with inorg. ^{32}P or $[\gamma\text{-}^{32}\text{P}]$ ATP. The approach is limited to specimens amenable to radiolabelling and poses certain safety and disposal problems. Alternatively, immunodetection with antibodies to the common phosphoamino acids may be employed, but the antibodies are relatively expensive and exhibit limited specificity and a certain degree of cross-reactivity. Pro-Q Diamond dye is a new fluorescent phosphosensor technol. suitable for the detection of phosphoserine-, phosphothreonine- and phosphotyrosine-containing proteins directly in isoelec. focusing gels, SDS-polyacrylamide gels and two-dimensional gels. Addnl., the technol. is appropriate for the detection of phosphoproteins or phosphopeptides arrayed on protein chips or affixed to beads. Dye-stained proteins and peptides can be excited with a laser-based light source of 532 or 543 nm or with a xenon-arc lamp-based system equipped with appropriate band pass filters. Alternatively, UV light of about 302 nm may be employed, providing that sufficiently long exposure times are used to collect the fluorescence signal. Pro-Q Diamond dye emits maximally at approx. 580 nm. The fluorescence-based detection technol. is easy to conduct, cost effective and allows rapid large-scale screening of protein and peptide phosphorylation in a variety of solid-phase assay formats.

RE.CNT 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

AN 1997:213477 CAPLUS

DN 126:273770

TI *Xenopus* actin depolymerizing factor/cofilin (XAC) is responsible for the turnover of actin filaments in *Listeria monocytogenes* tails

AU Rosenblatt, Jody; Agnew, Brian J.; Abe, Hiroshi; Bamberg, James R.; Mitchison, Timothy J.

CS Department of Biochemistry, University of California, San Francisco, CA, 94143, USA

SO Journal of Cell Biology (1997), 136(6), 1323-1332

CODEN: JCLBA3; ISSN: 0021-9525

PB Rockefeller University Press

DT Journal

LA English

AB In contrast to the slow rate of depolymerization of pure actin in vitro, populations of actin filaments in vivo turn over rapidly. Therefore, the rate of actin depolymerization must be accelerated by one or more factors in the cell. Since the actin dynamics in *Listeria monocytogenes* tails bear many similarities to those in the lamellipodia of moving cells, we have used *Listeria* as a model system to isolate factors required for regulating the rapid actin filament turnover involved in cell migration. Using a cell-free *Xenopus* egg extract system to reproduce the *Listeria* movement seen in a cell, we depleted candidate depolymerization proteins and analyzed the effect that their removal had on the morphology of *Listeria* tails. Immunodepletion of *Xenopus* actin depolymerization factor (ADF)/cofilin (XAC) from *Xenopus* egg extracts resulted in *Listeria* tails that were approximately five times longer than the tails from undepleted extracts. Depletion of XAC did not affect the tail assembly rate, suggesting that the increased tail length was caused by an inhibition of actin filament depolymerization. Immunodepletion of *Xenopus* gelsolin had no effect on either tail length or assembly rate. Addition of recombinant wild-type XAC or chick ADF protein to XAC-depleted extracts restored the tail length to that of control extracts, while addition of mutant ADF S3E that mimics the phosphorylated, inactive form of ADF did not reduce the tail length. Addition of excess wild-type XAC to *Xenopus* egg extracts reduced the length of *Listeria* tails to a limited extent. These observations show that XAC but not gelsolin is essential for depolymerization of actin filaments that rapidly turn over in *Xenopus* extracts. We also show that while the depolymerization activities of XAC and *Xenopus* extract are effective at depolymerizing normal filaments containing ADP, they are unable to completely depolymerize actin filaments containing AMPPNP, a slowly hydrolyzable ATP analog. This observation suggests that the substrate for XAC is the ADP-bound subunit of actin and that the lifetime of a filament is controlled by its nucleotide content.

L10 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN
AN 1995:707889 CAPLUS
DN 123:105683
TI Reactivation of phosphorylated actin depolymerizing factor and
identification of the regulatory site
AU Agnew, Brian J.; Minamide, Laurie S.; Bamberg, James R.
CS Dep. Biochem. Mol. Biol., Colorado State Univ., Fort Collins, CO, 80523,
USA
SO Journal of Biological Chemistry (1995), 270(29), 17582-7
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
AB Actin depolymerizing factor (ADF) occurs naturally in two forms, one of which
contains a phosphorylated Ser and does not bind G-actin or
depolymerize F-actin. Removal of this phosphate in vitro by alkaline
phosphatase restores full F-actin depolymerizing activity. To identify the
phosphorylation site, [32P]pADF was purified and digested with
endoproteinase Lys-C. The digest contained only one 32P-labeled peptide.
Further digestion with endoproteinase Asp-N and mass spectrometric anal.
showed that this peptide came from the N terminus of ADF. Alkaline
phosphatase treatment of one Asp-N peptide (mass 753) converted it to a
peptide of mass 673, demonstrating that this peptide contains the
phosphate group. Tandem mass spectrometric sequence anal. of this peptide
identified the phosphorylated Ser as the encoded Ser3 (Ser2 in
the processed protein). HeLa cells, transfected with either chick
wild-type ADF cDNA or a cDNA mutated to code for Ala in place of Ser24 or
Thr25, express and phosphorylate the exogenous ADF. Cells also expressed
high levels of mutant ADF when Ser3 was deleted or converted to either Ala
or Glu. However, none of these mutants was phosphorylated,
confirming that Ser3 in the encoded ADF is the single in vivo regulatory
site.

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L2      1412 SEA ABB=ON  PLU=ON  L1(L) (ISOLAT# OR SEPARAT# OR CHROOMATOGRAPH
        Y)
L3      89 SEA ABB=ON  PLU=ON  L2 AND (GALLIUM OR GA OR IRON OR FE OR
        ALUMINUM OR AL)
L4      7 SEA ABB=ON  PLU=ON  L3 AND (METAL(L) CHELAT# OR BAPTA OR IDA OR
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L6      94 SEA ABB=ON  PLU=ON  ("GEE KYLE"/AU OR "GEE KYLE R"/AU)
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L7      25 SEA ABB=ON  PLU=ON  ("MARTIN VLADIMIR"/AU OR "MARTIN VLADIMIR
        V"/AU)
L8      126 SEA ABB=ON  PLU=ON  L5 OR L6 OR L7
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        D QUE L8 STAT
        D QUE L9 STAT
        D BIB ABS
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        D 1-9 BIB ABS

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